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<b>(21) International Application Number:</b> PCT/US98/02494 <b>(22) International Filing Date:</b> 9 February 1998 (09.02.98)  <b>(30) Priority Data:</b> 60/037,561 11 February 1997 (11.02.97) US  <b>(71) Applicant (for all designated States except US):</b> MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> DUONG, Le, T. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). RODAN, Gideon, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).  <b>(74) Common Representative:</b> MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		<b>(81) Designated States:</b> CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> PROTEIN TYROSINE KINASE 2 (PYK2), NUCLEIC ACIDS, AND ASSAY  <b>(57) Abstract</b>  This invention is directed to nucleic acids encoding protein tyrosine kinase 2 (PYK2), to murine PYK2, to methods of making this protein using the nucleic acids, and to assays for inhibitors of PYK2.		

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## TITLE OF THE INVENTION

PROTEIN TYROSINE KINASE 2 (PYK2), NUCLEIC ACIDS, AND  
ASSAY

## 5 BRIEF DESCRIPTION OF THE INVENTION

This invention is directed to nucleic acids encoding protein tyrosine kinase 2 (PYK2), to murine PYK2, to methods of making this protein using the nucleic acids, and to assays for inhibitors of PYK2.

## 10 BACKGROUND OF THE INVENTION

Protein tyrosine kinase 2 (PYK2), also known as Cell Adhesion Kinase  $\beta$  (CAK $\beta$ ) and Related Adhesion Focal Tyrosine Kinase (RAFTK) is a recently described member of the focal adhesion kinase family (Avraham *et al.*, 1995 *J. Biol. Chem.* 270:27742-27751; Lev *et al.*, 15 1995 *Nature*. 376:737-745; and Sasaki, *et al.*, 1995 *J. Biol. Chem.* 270:21206-21219.). PYK2 was first cloned from human brain as a Grb-2 binding protein, and has also been cloned from rat and human brain libraries. There have been conflicting reports as to its cellular expression. In one study, abundant PYK2 transcripts were found in 20 brain and lower levels were detected in the kidney. In another report, PYK2 expression was also found to be most abundant in rat brain, but its transcripts could also be detected in kidney, spleen, lung, intestine and epididymis. PYK2 transcripts were also detected in rat fibroblast 3Y1 and WFB cell lines, as well as in the human T cell leukemia Jurkat line. 25 When cloned from the human megakaryocytic CMK cell line and from mouse brain, it was found to have wider tissue distribution beyond brain, notably spleen, lung, thymus and peripheral blood leukocytes. In addition, expression of PYK2 was reported in human CD34+ marrow cells, megakaryocytes and platelets.

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## DETAILED DESCRIPTION OF THE INVENTION

One aspect of this invention is are nucleic acids, substantially free from associated nucleic acids, which encode murine protein tryosine kinase 2 (PYK2). In one embodiment, the nucleic acid  
5 which encodes PYK2 is a DNA.

Another aspect of this invention is murine PYK2 cDNA, Murine PYK2 DNA is set forth in Figure 1 (SEQ ID NO:5).

Yet another aspect of this invention is murine PYK2 which is free from associated murine proteins. One murine PYK2 is set forth  
10 in Figure 1 (SEQ ID NO:6).

Another aspect of this invention is a method of making PYK2 by introducing nucleic acids into a cell, the nucleic acids comprising nucleic acids which encode PYK2, under conditions which transcription and translation of PYK2 occur. It is preferred that the  
15 nucleic acids be present in a vector, such as a plasmid or baculovirus vector. It is also preferred that the nucleic acids be under the control of transcriptional control elements, such as promoters and optionally enhancers. Such control elements are well known in the art.

Host cells which express PYK2 are also part of this  
20 invention. Preferred host cells include mammalian cells, insect cells, yeast and bacterial cells such as *E. coli*. Cell lines which permanently (rather than transiently) express murine PYK2 are also another aspect of this invention.

The recombinant PYK2, which is made using the cloning  
25 process of this invention may be used in assays in order to further characterize the biological function of PYK2 and to identify compounds such as agonists and antagonists which modulate its activity. A further aspect of this invention is an assay for the identification of compounds which modulate the activitiy of PYK2, and particularly inhibitors of  
30 PYK2 activity. This assay comprises the steps of: contacting recombinant PYK2 with a tyrosine substrate in the presence of radiolabeled ATP and a putative activity-modifying compound, and measuring the amount of radiolabeled tyrosine which is formed; and optionally comparing the amount of radiolabeled tyrosine formed in the

presence of the putative activity-modifying compound with that formed in the absence of the putative activity-modifying compound.

Integrins are the major family of cell surface receptors that mediate adhesive interactions, either to adjacent cells or to the extracellular matrix. Integrin signalling is mediated through the focal adhesion kinase (FAK) family of proteins. PYK2 is a member of the FAK family, and is involved in integrin-mediated signal transduction pathways in megakaryocytes, brain tissue and hematopoietic cells. Modulators of PYK2 would therefore be potential therapeutic agents for modulating platelet levels.

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is the cDNA sequence of mouse PYK2 and the deduced protein sequence. Intron sequences are in lower case letters. The exon sequence is capitalized. The boxed sequence of the deduced protein indicates the kinase domain. The circled prolines of the deduced protein indicate the proline rich domain.

As used throughout the specification and claims, the following definitions apply:

"PYK2" means protein tyrosine kinase 2, allelic variations of protein tyrosine kinase 2, and mutations or fragments thereof which retain at least about 85%, and preferably at least about 90% of the biological activity of native PYK2.

"Native PYK2" means the protein tyrosine kinase which is naturally occurring in an organism.

"Substantially free from associated nucleic acids" means that in a sample, there is less than about 5% (by weight) nucleic acids present which are other than nucleic acids encoding PYK2.

"Substantially free from associated murine proteins" means that in a sample, there is less than about 5% (by weight) protein which is other than murine PYK2.

"FAK" means focal adhesion kinase.

"Heterologous" PYK2 nucleic acid means that the nucleic acid was introduced to the cell, without regard as to whether the nucleic acid is from the same species as the cell; alternatively it refers to nucleic

acids encoding PYK2 in a cell whose ancestor had PYK2 introduced into the cell.

"Heterologous" PYK2 protein means that the PYK2 was encoded by a heterologous nucleic acid.

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FAK proteins, which are involved in cell adhesion processes, were not detected in a number of macrophage cell lines and it was therefore hypothesized that another cell adhesion-dependent kinase, homologous to FAK, may assume its function in these cells. PYK2 was recently identified as another member of the FAK family and its expression was detected in spleen, thymus, lung and peripheral blood leukocytes (Avraham, *et al.*, 1995 *supra*; Sasaki, *et al.*, 1995 *supra*). To evaluate PYK2 as a possible adhesion-dependent kinase in macrophages, specific probes were generated for PYK2 and FAK which were used to examine the expression of PYK2 and FAK in mouse tissues. As previously reported, for other species, PYK2 is highly expressed in brain and spleen, and at lower levels in kidney, lung and liver and has a more restricted tissue distribution than FAK.

Using a PYK2 probe, the full length cDNA was cloned from a mouse spleen cDNA library. The deduced amino acid sequence of the full length clone was found to be identical to the recently published amino acid sequence of the mouse RAFTK (Avraham, *et al.*, 1995, *supra*).

In addition, the full length FAK was cloned from a mouse osteoblastic MB1.8 cell line (Wesolowski, *et al.*, 1995, *Exp. Cell Res.*, 219: 679-686.).

PYK2 and FAK cDNAs were subsequently transfected into human embryonic kidney (HEK) 293 cells. Cell lines which permanently express either PYK2 or FAK were established and the expression levels of the exogeneously expressed mouse kinases were assessed by northern analysis.

The following Examples are presented to better illustrate the invention.

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#### EXAMPLE 1

### Cell Culture

Macrophages were induced by thioglycolate injection into the peritoneal cavities of adult BALB/c mice. After 4 days, cells were collected, washed and cultured in RPMI 1640 medium containing 10% FBS. After 3h at 37°C, the cultures were washed extensively to remove non-adherent cells and cultured overnight before samples were prepared for immunoprecipitation. Bone marrow derived macrophages were prepared as described by Li and Chen, 1995 *J. Leuk. Biol.* 57:484-490, which is hereby incorporated by reference. Non adherent cells were cultured in RPMI completed medium in the presence of human macrophage colony-stimulating factor (MCS-F, 250 units/ml, Genetics Institute, Cambridge, MA). Differentiated macrophages were prepared for immunoprecipitation after 5 days in culture.

Bone marrow derived osteoclast-like cells were prepared as described by Wesolowski, *et al.*, 1995 *Exp. Cell Res.* 219:679-686, which is hereby incorporated by reference. After collagenase-dispase treatment, mononucleated tartrate resistant phosphatase positive cells were released from the tissue culture plate using 30 nM echistatin (Merck Res. Labs., West Point, PA). Freshly isolated osteoclast-like cells were immediately solubilized in immunoprecipitation buffer.

### EXAMPLE 2

#### cDNA Cloning and Expression of mouse PYK2

Specific probes for mouse PYK2 and FAK were initially generated based on the non-homologous region between the proteins, which is adjacent to the C-terminal of the kinase domain. Using polymerase chain reaction (PCR), a specific probe for PYK2 (570bp) was generated using the 5'-primer AGTGA CATT T ATCAG ATGGA G (SEQ. ID. NO:1) and the 3'-primer GAATG GACTG TGCAC CGAGC C (SEQ. ID. NO:2) with cDNAs of mouse bone marrow derived osteoclast-like cells as template (Wesolowski, *et al.*, 1995, *supra*).

Similarly, a specific probe for FAK (700bp) was generated using the following primers: 5'- CAGCA CACAA TCCTG GAGGA G

(SEQ. ID. NO:3) and 3'- GCTGA AGCTT GACAC CCTCA T (SEQ. ID. NO. 4) with cDNAs of mouse osteoblastic MB1.8 cells as template (Wesolowski, *et al.*, 1995, *supra*). These probes were confirmed by sequencing analysis. PYK2 cDNA fragments were cloned from a mouse spleen  $\lambda$ -ZAP II cDNA library (Stratagene, La Jolla, CA) using the specific PYK2 probe. Full length PYK2 cDNA were constructed by ligation of two overlapping clones at the VspI site. The amino acid sequence of the isolated PYK2 cDNA clone was identical to the previously published mouse RAFTK sequence (Avraham, *et al.*, 1995 *supra*). Full length FAK cDNA was generated by PCR according to the published sequence as described in Hanks, *et al.*, 1992 *Proc. Natl. Acad. Sci. USA*. 89:8487-8491.

Both PYK2 and FAK cDNAs were subcloned into pCDNA3 plasmid (InVitrogen, San Diego, CA) and transfected into human embryonic kidney (HEK) 293 cells (ATCC, Rockland, MD) by electroporation at 200V, 960  $\mu$ F using a GenePulser (Biorad Labs, Richmond, CA). HEK 293 cells were subsequently subjected to G418 selection (800  $\mu$ g/ml, Gibco BRL) and clones were picked after 3 weeks in selection medium.

Expression of PYK2 and FAK in HEK293 cells were confirmed by Northern analysis using the respective probes, and Western blots were performed using anti-PYK2 antibodies. Mouse multiple tissue Northern blot was purchased from Clontech (Palo Alto, CA) and hybridization of the Northern blot using probes specific for PYK2, FAK and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were performed as described previously (Wesolowski, *et al.*, 1995, *supra*).

## EXAMPLE 3

Production and Affinity Purification of Polyclonal Antibodies  
to mouse PYK2

5           The PYK2 C-terminal domain (from Methionine residue 685 to end) was amplified by PCR using the mouse PYK2 as template. Amplified product was cloned into plasmid pGEX-4T (Pharmacia Biotech., Piscataway, NJ) and transformed in *E.coli* XL1-Blue (Stratagene). Expression of GST-PYK2 C-terminal fragment was  
10 induced using 0.5 mM IPTG, purified and cleaved from GST with thrombin, essentially according to the instructions of the manufacturer (Pharmacia). The purified C-terminal fragment of mouse PYK2 was used to immunize two rabbits (Research Genetics, Huntsville, AL) and the titers of both antisera were initially determined by ELISA using the  
15 recombinant C-terminal fragment of PYK2. Specificity of the immune sera was subsequently determined by Western blot by comparison to the preimmune sera. Polyclonal antibodies were then affinity purified by passing the combined fractions of both antisera through an affinity column, which was constructed using the same purified antigen cross  
20 linked to CNBr-activated Sepharose 4B according to the instructions of the manufacturer (Pharmacia).

          The antibodies were eluted from the column using 0.2 M Glycine, pH 2.5 and 1mM EGTA and the eluted fraction was then dialyzed against PBS containning 0.02% azide. Anti-PYK2 antibodies  
25 were stored at -70°C at a concentration of 0.5mg/ml.

## EXAMPLE 4

In vitro Kinase Assay

30           After cell attachment to ECM, IC-21 cells were solubilized in TNE lysis buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 1mM EDTA, 10% glycerol, 50 mM NaF, 1 mM sodium vanadate and protease inhibitors as described above. PYK2 were immunoprecipitated from the clarified lysates, half of the sample was  
35 subjected to immunoblotting with anti PYK2 antibodies, as described

above, and the other half was washed 2 times with the same lysis buffer, and with kinase assay buffer (1X) containing 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 10 mM MnCl<sub>2</sub> and 1 mM dithiothreitol. After removal of the wash buffer, 50 µl of kinase assay buffer containing 5 µCi [ $\gamma$ -<sup>32</sup>P] ATP (3000 Ci/mmol, Amersham), 10 mM ATP, 0.1% BSA and 100 µg of poly (Glu,Tyr) (molar ratio 4:1; Sigma) was added to the beads and incubated for 10 min at 30°C (Howell and Cooper, 1995 *Mol. Cell. Biol.* 14:5402-5411). The reaction mixtures (25 µl) were added to 25 µl of 30% trichloroacetic acid (TCA) and 0.1 M sodium pyrophosphate, followed by incubation at 4°C for 15 min. The precipitated proteins were transferred to a Multiscreen-FC filter plate (Millipore, Marlborough, MA), washed with ice cold 15% TCA (3X), allowed to dry and incorporation of <sup>32</sup>P into the substrate was counted on a Packard top count microplate scintillation counter (Packard, Meriden, CT). Each assay were performed as triplicate. The specific activity was determined by comparing the radioactive counts with immunoblot signals.

SEQUENCE LISTING

(1) GENERAL INFORMATION

- (i) APPLICANT: DUONG, LE T.  
RODAN, GIDEON A.
- (ii) TITLE OF THE INVENTION: PROTEIN TYROSINE KINASE 2  
(PYK2), NUCLEIC ACIDS AND ASSAY
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
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  - (C) CITY: Rahway
  - (D) STATE: NJ
  - (E) COUNTRY: USA
  - (F) ZIP: 07065-0900
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Diskette
  - (B) COMPUTER: IBM Compatible
  - (C) OPERATING SYSTEM: DOS
  - (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 60/037,561
  - (B) FILING DATE: 11-FEB-1997
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Sabatelli, Anthony D
  - (B) REGISTRATION NUMBER: 34,714
  - (C) REFERENCE/DOCKET NUMBER: 19792
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 732-594-1935
  - (B) TELEFAX: 732-594-4720
  - (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGTGACATTT ATCAGATGGA G

21

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAATGGACTG TGCACCGAGC C

21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CAGCACACAA TCCTGGAGGA G

21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCTGAAGCTT GACACCCTCA T

21

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3981 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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CCAGTCACAG GACTCTGTGT TTTATGGAAC TGAGTGCCAC AGGAAGAAGC AGAGAGTCGG 3780
AGGTCAGAAT GGACTTTGTG CCCTTCCTGC GTTCTCTTC TCCCTCTTTC CTCTCTCCCT 3840
CTTTCTTAC GTCTCCTTT TCTCCTCCC CTTTTCACAT CTGCTCCCT CCTCTCTCAT 3900
GTCTGTGGAG AACATTACC TTCCTCTTT TTGATCGGTG GTTGAATTAA AATTATTACC 3960
ATTTGCTTTG TGGCTCGTGC C 3981

```

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1009 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Ser Gly Val Ser Glu Pro Leu Ser Arg Val Lys Val Gly Thr Leu
 1          5          10          15
Arg Arg Pro Glu Gly Pro Pro Glu Pro Met Val Val Val Pro Val Asp
          20          25          30
Val Glu Lys Glu Asp Val Arg Ile Leu Lys Val Cys Phe Tyr Ser Asn
          35          40          45
Ser Phe Asn Pro Gly Lys Asn Phe Lys Leu Val Lys Cys Thr Val Gln
          50          55          60
Thr Glu Ile Gln Glu Ile Thr Ser Ile Leu Ser Gly Arg Ile
          65          70          75          80
Gly Pro Asn Ile Gln Leu Ala Glu Cys Tyr Gly Leu Arg Leu Lys His
          85          90          95
Met Lys Ser Asp Glu Ile His Trp Leu His Pro Gln Met Thr Val Gly
          100          105          110
Glu Val Gln Asp Lys Tyr Glu Cys Leu His Val Glu Ala Glu Trp Arg
          115          120          125
Tyr Asp Leu Gln Ile Arg Tyr Leu Pro Glu Asp Phe Met Glu Ser Leu
          130          135          140
Lys Glu Asp Arg Thr Thr Leu Leu Tyr Phe Tyr Gln Gln Leu Arg Asn
          145          150          155          160
Asp Tyr Met Gln Arg Tyr Ala Ser Lys Val Ser Glu Gly Met Ala Leu
          165          170          175
Gln Leu Gly Cys Leu Glu Leu Arg Arg Phe Phe Lys Asp Met Pro His
          180          185          190
Asn Ala Leu Asp Lys Lys Ser Asn Phe Glu Leu Leu Glu Lys Glu Val
          195          200          205
Gly Leu Asp Leu Phe Phe Pro Lys Gln Met Gln Glu Asn Leu Lys Pro
          210          215          220
Lys Gln Phe Arg Lys Met Ile Gln Gln Thr Phe Gln Gln Tyr Ala Ser
          225          230          235          240

```

Leu	Arg	Glu	Glu	Glu	Cys	Val	Met	Lys	Phe	Phe	Asn	Thr	Leu	Ala	Gly
				245					250					255	
Phe	Ala	Asn	Ile	Asp	Gln	Glu	Thr	Tyr	Arg	Cys	Glu	Leu	Ile	Gln	Gly
		260						265					270		
Trp	Asn	Ile	Thr	Val	Asp	Leu	Val	Ile	Gly	Pro	Lys	Gly	Ile	Arg	Gln
	275						280					285			
Leu	Thr	Ser	Gln	Asp	Thr	Lys	Pro	Thr	Cys	Leu	Ala	Glu	Phe	Lys	Gln
	290					295					300				
Ile	Arg	Ser	Ile	Arg	Cys	Leu	Pro	Leu	Glu	Glu	Thr	Gln	Ala	Val	Leu
305					310					315				320	
Gln	Leu	Gly	Ile	Glu	Gly	Ala	Pro	Gln	Ser	Leu	Ser	Ile	Lys	Thr	Ser
				325					330					335	
Ser	Leu	Ala	Glu	Ala	Glu	Asn	Met	Ala	Asp	Leu	Ile	Asp	Gly	Tyr	Cys
		340						345					350		
Arg	Leu	Gln	Gly	Glu	His	Lys	Gly	Ser	Leu	Ile	Met	His	Ala	Lys	Lys
	355					360						365			
Asp	Gly	Glu	Lys	Arg	Asn	Ser	Leu	Pro	Gln	Ile	Pro	Thr	Leu	Asn	Leu
	370				375						380				
Glu	Ala	Arg	Arg	Ser	His	Leu	Ser	Glu	Ser	Cys	Ser	Ile	Glu	Ser	Asp
385					390					395				400	
Ile	Tyr	Ala	Glu	Ile	Pro	Asp	Glu	Thr	Leu	Arg	Arg	Pro	Gly	Gly	Pro
				405					410					415	
Gln	Tyr	Gly	Val	Ala	Arg	Glu	Glu	Val	Val	Leu	Asn	Arg	Ile	Leu	Gly
		420						425					430		
Glu	Gly	Phe	Gly	Glu	Val	Tyr	Glu	Gly	Val	Tyr	Thr	Asn	His	Lys	
	435					440					445				
Gly	Glu	Lys	Ile	Asn	Val	Ala	Val	Lys	Thr	Cys	Lys	Lys	Asp	Cys	Thr
	450				455					460					
Gln	Asp	Asn	Lys	Glu	Lys	Phe	Met	Ser	Glu	Ala	Val	Ile	Met	Lys	Asn
465					470					475				480	
Leu	Asp	His	Pro	His	Ile	Val	Lys	Leu	Ile	Gly	Ile	Ile	Glu	Glu	Glu
				485					490					495	
Pro	Thr	Trp	Ile	Ile	Met	Glu	Leu	Tyr	Pro	Tyr	Gly	Glu	Leu	Gly	His
		500						505					510		
Tyr	Leu	Glu	Arg	Asn	Lys	Asn	Ser	Leu	Lys	Val	Pro	Thr	Leu	Val	Leu
	515					520						525			
Tyr	Thr	Leu	Gln	Ile	Cys	Lys	Ala	Met	Ala	Tyr	Leu	Glu	Ser	Ile	Asn
	530					535				540					
Cys	Val	His	Arg	Asp	Ile	Ala	Val	Arg	Asn	Ile	Leu	Val	Ala	Ser	Pro
545					550					555				560	
Glu	Cys	Val	Lys	Leu	Gly	Asp	Phe	Gly	Leu	Ser	Arg	Tyr	Ile	Glu	Asp
			565					570						575	
Glu	Asp	Tyr	Tyr	Lys	Ala	Ser	Val	Thr	Arg	Leu	Pro	Ile	Lys	Trp	Met
		580						585					590		
Ser	Pro	Glu	Ser	Ile	Asn	Phe	Arg	Arg	Phe	Thr	Thr	Ala	Ser	Asp	Val
	595					600						605			
Trp	Met	Phe	Ala	Val	Cys	Met	Trp	Glu	Ile	Leu	Ser	Phe	Gly	Lys	Gln
	610					615					620				
Pro	Phe	Phe	Trp	Leu	Glu	Asn	Lys	Asp	Val	Ile	Gly	Val	Leu	Glu	Lys
625					630					635				640	
Gly	Asp	Arg	Leu	Pro	Lys	Pro	Glu	Leu	Cys	Pro	Pro	Val	Leu	Tyr	Thr
			645						650					655	
Leu	Met	Thr	Arg	Cys	Trp	Asp	Tyr	Asp	Pro	Ser	Asp	Arg	Pro	Arg	Phe
		660						665					670		
Thr	Glu	Leu	Val	Cys	Ser	Leu	Ser	Asp	Ile	Tyr	Gln	Met	Glu	Lys	Asp
		675					680					685			

Ile Ala Ile Glu Gln Glu Arg Asn Ala Arg Tyr Arg Pro Pro Lys Ile  
 690 695 700  
 Leu Glu Pro Thr Thr Phe Gln Glu Pro Pro Pro Lys Pro Ser Arg Pro  
 705 710 715 720  
 Lys Tyr Arg Pro Pro Gln Thr Asn Leu Leu Ala Pro Lys Leu Gln  
 725 730 735  
 Phe Gln Val Pro Glu Gly Leu Cys Ala Ser Ser Pro Thr Leu Thr Ser  
 740 745 750  
 Pro Met Glu Tyr Pro Ser Pro Val Asn Ser Leu His Thr Pro Pro Leu  
 755 760 765  
 His Arg His Asn Val Phe Lys Arg His Ser Met Arg Glu Glu Asp Phe  
 770 775 780  
 Ile Arg Pro Ser Ser Arg Glu Glu Ala Gln Gln Leu Trp Glu Ala Glu  
 785 790 795 800  
 Lys Ile Lys Met Lys Gln Val Leu Glu Arg Gln Gln Lys Gln Met Val  
 805 810 815  
 Glu Asp Ser Gln Trp Leu Arg Arg Glu Glu Arg Cys Leu Asp Pro Met  
 820 825 830  
 Val Tyr Met Asn Asp Lys Ser Pro Leu Thr Pro Glu Lys Glu Ala Gly  
 835 840 845  
 Tyr Thr Glu Phe Thr Gly Pro Pro Gln Lys Pro Pro Arg Leu Gly Ala  
 850 855 860  
 Gln Ser Ile Gln Pro Thr Ala Asn Leu Asp Arg Thr Asp Asp Leu Val  
 865 870 875 880  
 Tyr His Asn Val Met Thr Leu Val Glu Ala Val Leu Glu Leu Lys Asn  
 885 890 895  
 Lys Leu Gly Gln Leu Pro Pro Glu Asp Tyr Val Val Val Val Lys Asn  
 900 905 910  
 Val Gly Leu Asn Leu Arg Lys Leu Ile Gly Ser Val Asp Asp Leu Leu  
 915 920 925  
 Pro Ser Leu Pro Ala Ser Ser Arg Thr Glu Ile Glu Gly Thr Gln Lys  
 930 935 940  
 Leu Leu Asn Lys Asp Leu Ala Glu Leu Ile Asn Lys Met Lys Leu Ala  
 945 950 955 960  
 Gln Gln Asn Ala Val Thr Ser Leu Ser Glu Asp Cys Lys Arg Gln Met  
 965 970 975  
 Leu Thr Ala Ser His Thr Leu Ala Val Asp Ala Lys Asn Leu Leu Asp  
 980 985 990  
 Ala Val Asp Gln Ala Lys Val Val Ala Asn Leu Ala His Pro Pro Ala  
 995 1000 1005  
 Glu  
 1

## WHAT IS CLAIMED IS:

1. A nucleic acid, free from associated nucleic acids which encodes murine protein tyrosine kinase 2 (PYK2).  
5
2. A nucleic acid according to Claim 1 which is DNA.
3. Murine PYK2 cDNA.
- 10 4. Murine PYK2 cDNA which is set forth in Figure 1.
5. A cell line comprising heterologous PYK2, and which expresses PYK2.
- 15 6. An assay to identify compounds which alter the activity of PYK2 comprising:
  - a) contacting recombinant PYK2 with a tyrosine substrate in the presence of radiolabeled ATP and a putative activity-modifying compound;
  - 20 b) measuring the amount of radiolabeled tyrosine which is formed; and
  - c) optionally comparing the amount of radiolabeled tyrosine formed in the presence of the putative activity-modifying compound with that formed in the absence of the putative activity-modifying compound.

TITLE OF THE INVENTION

PROTEIN TYROSINE KINASE 2 (PYK2), NUCLEIC ACIDS, AND  
ASSAY

5 ABSTRACT OF THE INVENTION

This invention is directed to nucleic acids encoding protein tyrosine kinase 2 (PYK2), to murine PYK2, to methods of making this protein using the nucleic acids, and to assays for inhibitors of PYK2.

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attcggcgccgctcgacctcagcctctgcaggcagagccgcgcgtcctacctgcggcggc 60  
 tgcgtcacctggccagcccgagccctggcccgagtcgcgcgcctcgcgcgaggactg 120  
 caatgtcccggtcctagctgcagtctgagaggATGCCGGGTGCTGAGCCCTTGAGCC 180  
 M S G V S E P L S R 10  
 GTGTAAGTGGGCACCTTACGCCGGCCTGAGGGCCCCCAGAGCCCATGGTGGGTAC 240  
 V K V G T L R R P E G P P E P M V V P 30  
 CAGTGGATGTGGAGAGGAAGACGTGCGCATCCTCAAGGTCTGTCTTACAGCAACAGCT 300  
 V D V E K E D V R I L K V C F Y S N S F 50  
 TCAACCCAGGGAAGAACTTCAAGCTTGTCAAAATGCACAGTGCAGACAGAGATCCAGGAGA 360  
 N P G K N F K L V K C T V Q T E I Q E I 70  
 TCATCACCTCCATCCTCCTGAGTGGCGAATAGGGCCCAACATCCAGCTGGCTGAATGCT 420  
 I T S I L L S G R I G P N I Q L A E C Y 90  
 ATGGGCTGAGGCTGAAGCACATGAAGTCAGACGAGATCCACTGGCTGCCACCCACAGATGA 480  
 G L R L K H M K S D E I H W L H P Q M T 110  
 CCGTGGCGGAAGTGCAGGACAAGTATGAATGTCTACACGTGGAAGCTGAGTGGAGGTATG 540  
 V G E V Q D K Y E C L H V E A E W R Y D 130  
 ACCTTCAAATCCGCTACTTGCCGGAAGACTTCATGGAGAGCCTGAAGAAGACAGGACCA 600  
 L Q I R Y L P E D F M E S L K E D R T T 150  
 CATTGCTGTACTTTATCAACAGCTCCGGAATGACTACATGCAACGCTACGCCAGCAAGG 660  
 L L Y F Y Q Q L R N D Y M Q R Y A S K V 170  
 TCAGTGAAGGCATGGCTCTGCAGCTGGGCTGTCTGGAGCTCAGGAGATTCTTCAAGGACA 720  
 S E G M A L Q L G C L E L R R F F K D M 190

FIG. 1A

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780 TGGCCCAATGCACTGGACAAAAGTCCAACTTTGAACTCCTGGAAAAAGATCGGTC  
210 P H N A L D K K S N F E L L E K E V G L  
840 TGGACCTGTTTCCCAAAGCAGATGCAGGAAACTTAAAGCCCAAGCAGTCCGGAAGA  
230 D L F F P K Q M Q E N L K P K Q F R K M  
900 TGATCCAGCAGACCTTCCAGCAGTATGCATCACTCCGGGAGGAGAGTGTGTCAATGAAAT  
250 I Q Q T F Q Q Y A S L R E E C V M K F  
960 TCTTCAATACCTAGGGGCTTTGCCAACATTGACCAGGAGACCTACCGCTGCCGAACCTCA  
270 F N T L A G F A N I D Q E T Y R C E L I  
1020 TTCAAGGATGGAACATTACTGTGGACCTGTGTCACTCGGCCCTAAAGGCATCCGTCAGCTGA  
290 Q G W N I T V D L V I G P K G I R Q L T  
1080 CAAGTCAAGATACAAAGCCACCTGCTGGCCGAGTTTAAAGCAGATCAGATCCATCAGGT  
310 S Q D T K P T C L A E F K Q I R S I R C  
1140 GCCTCCCATTTGGAAGAGACCCAGGCAGTCTCTGCAGCTGGGCATCGAGGGTGCCCCCAGT  
330 L P L E E T Q A V L Q L G I E G A P Q S  
1200 CCTTGCTATCAAAACGTCGTCCTTGGCAGAGGCTGAGAACATGGCTGATCTCATAGATG  
350 L S I K T S S L A E A E N M A D L I D G  
1260 GCTACTGCAGGCTGCAAGGAGAACATAAGGGCTCTCTCATCATGCTGCCAAGAAAGATG  
370 Y C R L Q G G E H K G S L I M H A K K D G  
1320 GTGAGAAGGAACAGCCTGCTCAGATCCCCACACTAAACCTGGAGGCTCGGCGGTCGC  
390 E K R N S L P Q I P T L N L E A R R S H  
1380 ACCTCTCAGAAAGCTGCAGCATAGAGTCAGACATCTATGCGGAGATCCCGATGAGACCC  
410 L S E S C S I E S D I Y A E I P D E T L

FIG. 1B

SUBSTITUTE SHEET (RULE 26)

WEST

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1440	TGCGAAGACCAGGAGTCCACAGTACGGGTGTTGCCCGTGAAGAAGTAGTTCTTAACCGCA
430	R R P G G P Q Y <span style="border: 1px solid black; padding: 0 2px;">G V A R E E V V L N R I</span>
1500	TTCTGGGTGAAGGCTTCTTTGGGGAGGTCTATGAAGGTGTCTACACGAACCAACAAAGGGG
450	<span style="border: 1px solid black; padding: 0 2px;">L G E G F F G E V Y E G V Y T N H K G E</span>
1560	AAAAAATTAATGTGGCCGTCAGACCTGTAAAGAAAGACTGTACCCAGGACAACAAGGAGA
470	<span style="border: 1px solid black; padding: 0 2px;">K I N V A V K T C K K D C T Q D N K E K</span>
1620	AGTTCATGAGTGAGGCAGTGATCATGAAGAATCTTGACCACCCCTCACATCGTGAAGCTGA
490	<span style="border: 1px solid black; padding: 0 2px;">F M S E A V I M K N L D H P H I V K L I</span>
1680	TTGGCATCATTTGAAGAGGAACCCACCTGGATTATCATGGAACTGTATCCTTATGGGGAGC
510	<span style="border: 1px solid black; padding: 0 2px;">G I I E E E P T W I I M E L Y P Y G E L</span>
1740	TGGGACACTACCTGGAACGAAATAAAACTCCCTGAAGGTACCCACTCTGGTCCCTGTACA
530	<span style="border: 1px solid black; padding: 0 2px;">G H Y L E R N K N S L K V P T L V L Y T</span>
1800	CCCTACAGATATGCAAGCCATGGCCCTATCTGGAGAGCATCAACTGTGTGCACAGGGATA
550	<span style="border: 1px solid black; padding: 0 2px;">L Q I C K A M A Y L E S I N C V H R D I</span>
1860	TTGCTGTCCGGAACATCCTGGTGGCCCTCTCCTGAGTGTGTGAAGCTGGGGGACTTTGGGC
570	<span style="border: 1px solid black; padding: 0 2px;">A V R N I L V A S P E C V K L G D F G L</span>

FIG.1C

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TCTCCGGTACATGAGGACGAAGACTATTACAAAGCCTCTGTGACACGTCTACCCATCA 1920  
 S R Y I E D E D Y Y K A S V T R L P I K 590  
 AATGGATGTCCCGAGTCCATCAACTTCCGCCGCTTCACAACCGCCAGTGATGCTCTGGA 1980  
 W M S P E S I N F R R F T T A S D V W M 610  
 TGTGTGCTGATGTCATGTGGAGATCCTCAGCTTTGGGAAGCAGCCTTCTTCTGGCTCG 2040  
 F - A V C M W E I L S F G K Q P F F W L E 630  
 AAAATAAGGATGTCATCGGAGTGCTGGAGAAAGGGACAGGCTGCCCAAGCCGAACTCT 2100  
 N K D V I G V L E K G D R L P K P E L C 650  
 GTCCGCCTGTCCTTTACACACTCATGACTCGCTGCTGGGACTACGACCCCGAGTGACCCGGC 2160  
 P P V L Y T L M T R C W D Y D P S D R P 670  
 CCGCTTCACGGAGCTTGTGTGAGCCTCAGTGACATTATCAGATGGAGAAGGACATG 2220  
 R F T E L V C S L S D I Y Q M E K D I A 690  
 CCATAGAGCAAGAAAGGAATGCTCGCTACCGACCCCTAAATATTGGAGCCTACTACCT 2280  
 I E Q E R N A R Y R (P) K I L E (P) T T F 710  
 TTCAGGAACCCCAAGCCAGCCGCGCCCAAGTACAGACCTCTCCACAGACCAACC 2340  
 Q E (P) (P) K (P) S R (P) K Y R (P) (P) Q T N L 730  
 TGCTGGCTCCTAAGCTGCAGTTCAGGTCCCTGAGGGTCTGTGTGCCAGCTCTCCTACGC 2400  
 L A (P) K L Q F Q V (P) E G L C A S S (P) T L 750  
 TTACCAGCCCTATGGAGTATCCATCTCCAGTTAACTCGCTGCACACCCACCTCTCCACC 2460  
 T S (P) M E Y (P) S (P) V N S L H T (P) (P) L H R 770  
 GGCACAATGTCTTCAAGCGCCACAGCATGCGGGAGGAGGACTTCATCCGGCCCGTAGCC 2520  
 H N V F K R H S M R E E D F I R P S S R 790

FIG.1D

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2580 GAGAAGAGGCCAGCAGCTCTGGGAGGCAGAGAAGATCAAGATGAAGCAGGTCCTAGAAA  
810 E E A Q Q L W E A E K I K M K Q V L E R  
2640 GACAGCAGAAGCAGATGGTGGAGATTCCAGTGCGTGAAGCGAGAGGAAAGATGCTTGG  
830 Q Q K Q M V E D S Q W L R R E E R C L D  
2700 ACCCTATGGTTATATGAATGACAAGTCCCCACTGACTCCAGAGAAGGAGCGCGGTACA  
850 P M V Y M N D K S P L T P E K E A G Y T  
2760 CGGAGTTCACAGGGCCCCACAGAAACCCCTCGGCTCGGTGCACAGTCCATTCAGCCCA  
870 E F T G P P Q K P P R L G A Q S I Q P T  
2820 CAGCCAACCTGGACAGGACCGATGACCTCGTGTACCAATGTATGACCCCTGGTGAGG  
890 A N L D R T D D L V Y H N V M T L V E A  
2880 CTGTGCTGGAACCTCAAGAACAGCTTGCGCCAGTTGCCCCCTGAGGACTATGTGTGGTGG  
910 V L E L K N K L G Q L P P E D Y V V V V  
2940 TGAAGAACGTGGGGCTGAACCTGCGGAAGCTCATCGGCAGTGTGGACGATCTCTTGCCCT  
930 K N V G L N L R K L I G S V D D L L P S  
3000 CCTTGCCGGCATCTTCGAGGACAGAGATTGAAGGGACCCAGAACTGCTCAACAAAGACC  
950 L P A S S R T E I E G T Q K L L N K D L  
3060 TGGCAGAGCTCATCAACAAGATGAAGTTGGCTCAGCAGAACGCCGTGACGTCCTGAGTG  
970 A E L I N K M K L A Q Q N A V T S L S E  
3120 AGGACTGCAAGCGGACATGCTCAGCGTCCCATACCTGGCTGTGGATGCCAAGAACC  
990 D C K R Q M L T A S H T L A V D A K N L  
3180 TGCTGGATGCTGTGGACCAAGCCAAGGTGTGGCTAATCTGGCCACCCGCTGCAGAGT  
L D A V D Q A K V V A N L A H P P A E \*

FIG. 1E

SUBSTITUTE SHEET (RULE 26)

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3240 gatcaagagagggccacctgcctgcattcttgcacccacctgtcttgccataaccttctc  
3300 ctgccttgcctttggttattggctctccagggaagctgagaagagtgccatcccccttgc  
3360 cactttgcacgacacccccctcttcccccaacccacccagactgtgctactcaggctgca  
3420 tctggacagaaaggactctgggcacagacacgggtgggtgacatagttcataggggta  
3480 ctactgccagccactccctcttaccacagcctgggtgctggagcatcatggggtcatg  
3540 agtgtacccctaacggccaagatggcttctgcattggacatttgagagccagtattcctc  
3600 ctctctcttcagccctcagggaacccctgatacacagaggggacagaggggtttatttgt  
3660 agagaagctggtgagatgagggctggacctggctctcttgtacagtgtacattgggaattt  
3720 attaatgtgagttgacctggatggacagccaaggccatagtcaggagcaaaccaat  
3780 ccagtcacaggactctgtgtttatggaactgagtgccacaggaagaaagcagagatcgg  
3840 aggtcagaatggactttgtgccccctcctgcgtttctcttctccctcttcccttccct  
3900 ctttctctacgtctccttttctcctcccccttttcacatctgctccccctctctcat  
3960 gtctgtggagaacatttaccttcttcttttgatcgggtggtgaattaaaattattacc  
3981 atttgctttgtggctcgtgcc

FIG. 1F

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/02494

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(6) : C12N 1/00, 5/10, 15/54; C12Q 1/48, 1/68 US CL : 536/23.2; 435/6, 252.3, 254.11, 325, 410 According to International Patent Classification (IPC) or to both national classification and IPC														
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 536/23.2; 435/4, 6, 7.4, 15, 194, 252.3, 254.11, 320.1, 325, 410 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Extra Sheet.														
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
X	AVRAHAM et al. Identification and Characterization of a Novel Related Adhesion Focal Tyrosine Kinase (RAFTK) from Megakaryocytes and Brain. Journal of Biological Chemistry. 17 November 1995, Vol. 270, No. 46, pages 27742-27751, especially Figure 2, row 2.	1-4												
Y		5-6												
Y	US 5,573,944 A (KIRSCHNER ET AL.) 12 November 1996, column 2, lines 52-63; column 7, lines 50-55; claims 20-23.	5												
Y	US 5,538,858 A (MALIA ET AL.) 23 July 1996, claims 1-3, 7, 10	6												
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.														
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>*A* document defining the general state of the art which is not considered to be of particular relevance</td> <td>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>*B* earlier document published on or after the international filing date</td> <td>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>*A* document member of the same patent family</td> </tr> <tr> <td>*O* document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>*P* document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family	*O* document referring to an oral disclosure, use, exhibition or other means		*P* document published prior to the international filing date but later than the priority date claimed	
* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention													
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone													
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art													
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family													
*O* document referring to an oral disclosure, use, exhibition or other means														
*P* document published prior to the international filing date but later than the priority date claimed														
Date of the actual completion of the international search		Date of mailing of the international search report												
26 MARCH 1998		10 JUN 1998												
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer GABRIELE E. BUGAJSKY Telephone No. (703) 308-0196												

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/02494

### B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN-CAS FILES REGISTRY, MEDLINE, CAPLUS, N-GENESEQ, GENBANK/EMBL

search terms: protein tyrosine kinase, pyk2, pyk 2, mouse, murine, mus, rafk, cadtk, cakbeta, cell adhesion kinase  
beta, related adhesion focal tyrosine kinase, inhibit?, modulat?, antagoni?, agoni?